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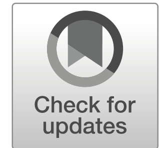
MICROBIAL ANTI-CANCER THERAPY AND PREVENTION (PJF RIDER, L SWEENY AND KG KOUSOULAS, SECTION EDITORS)



Anti-viral Immunity in the Tumor Microenvironment: Implications for the Rational Design of Herpes Simplex Virus Type 1 Oncolytic Virotherapy

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Abstract

Purpose of Review The design of novel herpes simplex type I (HSV-1)–derived oncolytic virotherapies is a balancing act between safety, immunogenicity, and replicative potential. We have undertaken this review to better understand how these considerations can be incorporated into rational approaches to the design of novel herpesvirus oncolytic virotherapies.

Recent Findings Several recent papers have demonstrated that enhancing the potential of HSV-1 oncolytic viruses to combat anti-viral mechanisms present in the tumor microenvironment leads to greater efficacy than their parental viruses.

Summary It is not entirely clear how the immunosuppressive tumor microenvironment affects oncolytic viral replication and spread within tumors. Recent work has shown that the manipulation of specific cellular and molecular mechanisms of immunosuppression operating within the tumor microenvironment can enhance the efficacy of oncolytic virotherapy. We anticipate that future work will integrate greater knowledge of immunosuppression in tumor microenvironments with design of oncolytic virotherapies.

Keywords HSV · Oncolytic · Herpesvirus · VC2

Introduction

The utility of genetically engineered HSV as "antineoplastic agents" was first demonstrated in 1991 [1] and led to the first FDA approval of a herpesvirus oncolytic agent in 2015. This first report used a virus created in the Knipe laboratory that possessed a small deletion in the middle one third of the HSV-1 thymidine kinase (tk) gene [2]. The tk mutation rendered the virus significantly impaired for replication in non-dividing cells such as neurons and was proposed to attenuate its neurovirulence potential. Since then, there have been many approaches to the development of HSV-derived viruses for oncolytic virotherapy [3–6].

T-VEC™ is the first FDA-approved oncolytic virus [4]. T-VEC™ is an attenuated herpes simplex virus-1 (HSV-1) derived from the JS-1 viral strain and engineered to have deletions in the genes encoding the infected cell protein (ICP) γ34.5 and ICP47 protein [7]. T-VEC™ also encodes the human cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF). GM-CSF functions as a potent stimulator of anti-tumor immunity by promoting the recruitment and maturation of macrophages and dendritic cells (DCs) [7]. The GM-CSF gene is inserted in place of the two deleted γ34.5genes. The γ34.5 protein, a major viral neurovirulence factor, is required for efficient viral replication in normal tissues [8]. γ34.5 reverses a cellular block to protein synthesis in infected cells [9]. HSV-1 infection leads to activation of double-stranded RNA-dependent protein kinase R (PKR) and activated PKR then phosphorylates eukaryotic initiation factor 2 alpha (eIF2α), significantly reducing viral and host protein synthesis in infected cells [10]. The γ34.5 protein reverses phosphorylation of eIF2α restoring protein synthesis

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[11]. Additionally, γ34.5 has been reported to have several other functions including blocking IFN-I responses and autophagy [10, 12–14]. The γ34.5 gene deletion has been included in T-VEC™ and the majority of HSV-1 oncolytic virotherapy (OVT) candidates [15]. The ICP47 gene functions to inhibit the transporter associated with antigen presentation (TAP) in HSV-1 infected cells [16] and blocks CD8+ T cell responses [17]. Inactivation of ICP47 was proposed to induce a more effective anti-tumor immune response [7]. Furthermore, the deletion of the ICP47 gene upregulates the expression of the unique short (US) 11 gene that, due to some functional redundancy, compensates γ34.5-deleted HSV-1 replication in tumor cells [7, 18, 19].

Mechanisms for increasing the safety of HSV-oncolytic vectors mainly include the selective replication/infection in cancer cells. Approaches to accomplish this include the following: (1) deletion or mutation of genes that facilitate replication in normal cells, while allowing replication in cancer cells [2, 20] which can compensate for the missing function; (2) targeting virus infection to specific cancer cell receptors [21–23]; (3) miRNA target sites engineered into HSV genes [24, 25]; and (4) transcriptional and translational control of essential viral gene expression [26, 27]. Many of these approaches also enhance safety by attenuating neurovirulence, a key aspect of herpes simplex pathogenesis [28]. More recently, novel determinants of neurovirulence including defects in entry into neurons [29•, 30–33], as well as retrograde and anterograde transport [34•] in neurons, are being incorporated into novel HSV-1-derived oncolytic viruses.

Many characteristics of herpesviruses inform their use as oncolytic virotherapy agents. These include the incredible amount of knowledge regarding the molecular biology of these viruses, the large coding capacity facilitating transgene expression, relative safety, and ease of genetic manipulation. With the approval of T-VEC in 2015, there has been an explosion of next-generation approaches to herpesvirus OVT. These next-generation candidates seek to address perceived shortcomings in the viral vector, and focus tropism, as well as use hypothesis-driven approaches to address the unique aspects of tumor microenvironments by arming viruses with additional transgenes that can modulate and enhance anti-tumor immune responses [5, 35, 36•].

The ease with which recombinant HSV can be created has led to a significant increase in the generation and testing of novel recombinant HSV for OVT [21, 35, 37, 38]. A critical aspect of the design of OVT vectors is balancing between viral attenuation for safety reasons and enhanced replication and immunogenicity [39]. T-VEC was derived from a clinical HSV-1 isolate and chosen for its superior ability to replicate and lyse in a number of cancer cell lines [7]. The parental JS1 strain was then attenuated with the γ34.5 and ICP47 deletions. As described above, these viral gene products play an extensive role in subverting anti-viral immunity. It is not entirely clear how these mutations affect the overall efficacy of HSV-1 OVT. Additionally, γ34.5 protein is a structural component of the virion particle, functions in assembly and egress, and its deletion reduces overall production of infectious virions [40, 41].

Interestingly, recent approaches have chosen to revert or more precisely engineer some of the mutations in T-VEC™ to achieve greater therapeutic efficacy [15, 42•]. This brief review will focus on recent work suggesting that, contrary to the past direction of HSV-oncolytic vector design, it may best serve this promising therapy to enable evasion of anti-viral immunity to boost their efficacy.

Anti-viral Responses and HSV-1 OVT Efficacy

Several recent reports support the argument that perhaps more attenuation is not better and that more effective oncolytic virotherapy agents will be those with greater replicative potential. To facilitate their replication and spread as well as lifelong infection of hosts, herpesviruses are prolific modulators of host immunity [43]. Restoration of TAP function in T-VEC™-infected cells was hypothesized to enhance its immunogenicity. However, this hypothesis was never formally tested. Contrarily, Pourchet et al. [42•] hypothesized that generating viruses that were better able to evade CD8+ anti-viral T cells may lead to greater anti-cancer benefit. To test this hypothesis, this group created an HSV-1-derived oncolytic virus that replaced γ34.5 genes with the bovine herpesvirus (BHV) UL49.5 and US11 genes under the control of an immediate early promoter (BV49.5, for a list of HSV OVT viruses discussed in this text, please refer to Table 1). UL49.5 and HSV ICP47 are functional homologs [47]. Both BHV UL49.5 and HSV ICP47 disrupt MHCI antigen processing and presentation via TAP disruption. However, while ICP47 competes for peptide binding, UL49.5 function leads to proteasomal degradation of TAP [47] and subsequent down-regulation of MHCI [48]. Mutations in the BHV UL49.5 domain required for TAP binding and degradation have been incorporated into a BHV vaccine reported to possess superior efficacy [49]. While HSV ICP47 does not inhibit murine TAP, BHV UL49.5 is capable of binding both murine and human TAP. Thus, using the BHV UL49.5 allowed them to test whether ICP47 makes a positive or negative contribution to the efficacy of the OVT in murine models. In a murine model of bladder cancer (MBT2), BV49.5 was compared to a virus that possessed a point mutation precluding expression of UL49.5 (BV49.5-FS) [42•]. BV49.5 reached higher titers in tumors and demonstrated greater reduction of tumor growth in injected and non-injected tumors than BV49.5-FS. Importantly, this greater efficacy was shown to be characterized by greater numbers of activated tumor-specific T cells and the benefit was abolished in mice in which CD8+ T cells

Table 1 HSV-1 oncolytic vectors discussed in this review

Oncolytic HSV-1	Strain	Mutations	Transgene inserted	Reference
T-VEC	JS-1	γ34.5Δ, ICP47Δ	Human GM-CSF	[7]
BV49.5	Patton	γ34.5Δ	BHV-1 UL49.5, IEUS11	[42•]
OV-CDH1	Q1	γ34.5Δ, ICP6Δ	Human *CDH1*	[36•]
rQNestin34.5	F	γ34.5Δ, ICP6Δ	Nestin-Hsp68promoter-γ34.5	[44]
RAMBO	F	γ34.5Δ, ICP6Δ	Human Vstat120	[45]
rRp450	KOS	ICP6Δ	Rat CYP2B1 gene	[46]

were depleted. Interestingly, when these viruses were altered to express murine GM-CSF, the GM-CSF expression did not result in detectable improvement. Further, in the 4T1 murine model of breast cancer, BV49.5 was more effective in reducing the number of metastases in the lungs, which was similarly dependent on UL49.5 expression and CD8+ T cells [42•].

It is not clear how exactly disruption of TAP by this virus endows it with greater ability to shrink tumors and facilitate the generation of anti-tumor T cells. Beyond better replication and spread in tumors which is expected to liberate greater numbers of tumor associated antigens (TAAs), the authors speculate that, based on what is known about antigen processing and presentation in tumors that are TAP deficient [50, 51], perhaps there is generation of unique anti-tumor T cells recognizing new T cell epitopes. Indeed, novel epitopes generated via immunotherapies including OVT has been shown to be correlated with efficacy [52, 53]. The authors note that it is also important to consider the high seroprevalence of HSV-1 in the human population [54]. It is expected that the majority of individuals receiving HSV-1-derived oncolytic virotherapy will be HSV-1 seropositive. While Pourchet et al. [42•] and others [7, 55] have demonstrated that there is no difference in OVT efficacy between mice that were pre-exposed to HSV-1, the maximum benefit of HSV-1-derived OVT may require the full complement of immune evasion gene products, such as ICP47, in seropositive individuals.

While the previous study analyzed the contribution of evasion of adaptive anti-viral immune responses via disruption of TAP, most studies have focused on innate immunity in the tumor microenvironment. A recent study used a novel oncolytic virus to evade NK cell immune responses in the tumor microenvironment and enhance the efficacy of HSV-1 OVT [36•]. The authors overexpressed E-cadherin using the HSV-1 pIE4/5 promoter in an HSV-1 backbone with deletions in γ34.5 and ICP6, the viral ribonucleotide reductase. E-cadherin is a cellular protein involved in cellular adherence, as well as acting as a ligand for the inhibitory NK cell receptor KLRG1+ [56]. Overexpression of E-cadherin on infected cells was hypothesized to fulfill two functions: (1) inhibit NK cell activity and (2) facilitate cell-to-cell spread by concentrating nectin-1, the HSV-1 entry receptor [57], and a cofactor in E-cadherin cell-cell adherence [58]. Glioblastoma cells (Gli36, U251, U87, GBM30) infected with the resulting virus, OV-CDH1, were less susceptible to lysis by KLRG1+ NK cells compared to cells infected with control virus. However, when KLRG1− cells were used, no differences in cell killing were detected [36•]. In vitro, OV-CDH1 exhibited enhanced plaque sizes in monolayers of human glioblastoma cell lines, GLi36 or U251, compared to control virus plaque sizes. Additionally, the expression of E-cadherin was shown to lead to improvement of viral entry and maximum viral titers. The in vivo efficacy of OV-CDH1 was evaluated using both xenograft (GBM30, U87dEGFR) and immunocompetent (Gl261N4) mouse models of glioblastoma. OV-CDH1 was superior to control viruses at reducing tumor rates of growth and providing significant survival benefit in all models tested. Further, in the immunocompetent model, viral titers of OV-CDH1 virus were one log greater than the control virus. Interestingly, while depletion of NK cells resulted in greater efficacy for both OV-CDH1 and control viruses, a significant difference between virus treatments remained suggesting that perhaps the ability of OV-CDH1 to achieve greater spread was a more important contributor to the efficacy of OV-CDH1 than NK cell evasion.

Several other recent reports in glioblastoma support the idea that early suppression of, at least, innate responses can positively affect the outcome of OVT. A single dose of TGFβ1 (intravenous) prior to OVT of nude mice bearing aggressive U87dEGFR tumors was shown to increase oncolytic HSV viral titers in tumors [59]. TGFβ is a pleiotropic, secreted cytokine that has been shown to be a significant contributor to immunosuppression in the tumor microenvironment [60]. In both xenograft (GB30) and syngeneic (4C8) glioblastoma mouse models, TGFβ1 treatment was found to decrease NK cell, macrophage, and microglia infiltration into tumors [59]. This reduction was correlated with reduced tumor growth rates and a significant increase in median survival time after OVT. While TGFβ1 is a broad spectrum suppressor involving multiple cell types, the group was able to replicate these findings with targeted depletion of NK cells using anti-asialo-GM1 antibody [59]. These findings suggest that the cell population targeted with TGFβ1 is NK cell.

In another recent study, in both a xenograft (U87dEGFR) and syngeneic (KR158dEGFR) models of glioblastoma, infiltration of activated NK cells began at two hours post treatment with HSV OVT (rQNestin34.5) [44]. Specific NK cell subsets expressing the activating NK cell receptors NKp30 and

NKp46 were identified as responsible for killing of HSV OVT-infected cells. This group showed that OVT efficacy and viral titers were significantly enhanced with antibody depletion of NK cells. Interestingly, it was further shown that NK cells orchestrate macrophage activation in this model, evidenced by attenuation of macrophage activation in NK-depleted animals.

A role for macrophage and microglia cells in anti-viral responses limiting viral replication and OVT efficacy has been reported in a number of studies. Addition of microglia to in vitro culture of U87 glioblastoma cells reduced viral growth and oncolytic potential [61]. This reduction was demonstrated to be the result of phagocytosis of virus by microglia and subsequent blocking of viral gene expression in these cells. The authors found that inhibition of viral replication after phagocytosis by microglia was dependent on STAT1 and STAT3 activities. Importantly, the treatment of mice with STAT inhibitors in the U87 GBM xenograft model led to increased viral replication and decreased tumor growth rates. Another recent study has identified a role for macrophage and microglia cells in limiting OVT efficacy in GBM [62]. In this study, inhibition of viral replication was shown to be mediated by TNFα secreted by macrophage or microglial cells in vitro. Importantly, this group demonstrated that inhibition of TNFα in an athymic U87dEGFR human GBM model increased viral replication which correlated with an increased survival benefit [62].

An interesting approach by Thorne et al., using The Cancer Genome Atlas (TCGA), reported that low levels of CCN1 mRNA were positively associated with better survival outcome in glioblastomas [63]. CCN1 is a secreted protein found in the tumor microenvironment and involved in upregulation of IFN-I responses and inflammation [64]. This group hypothesized that neutralization of CCN1 may have some therapeutic value during HSV-1 oncolytic virotherapy. Anti-CCN1 treatment of subcutaneous tumors led to decreased macrophage and NK cell tumor infiltration. Mechanistically, the group demonstrated that binding of CCN1 to integrins on the cell surface of macrophages upregulated the expression of pro-inflammatory genes. Anti-CCN1 treatment reversed much of the macrophage-mediated viral clearance in vitro. In subcutaneous glioblastoma tumors treated with anti-CCN1 prior to OVT, all mice demonstrated partial response or stable disease before progression as compared to two mice showing partial response in control groups.

A similar approach was taken with BAI1, a G protein–coupled receptor involved in adhesion, which was found to have reduced expression in many solid tumors [45]. BAI1 is expressed on macrophages and microglia, and is a pattern recognition receptor, and the extracellular portion modulates phagocytosis [65, 66]. This group hypothesized that expressing the extracellular fragment of BAI1 (Vstat120) using an oncolytic herpesvirus vector (RAMBO) could interfere with the anti-viral activity of BAI1 [45]. In an athymic model of glioblastoma (U87dEGFR) treated with RAMBO, they reported decreased numbers and activation of infiltrating macrophages compared to those treated with control virus rHSVQ1. Further, the presence of Vstat120 rescued the suppression of viral replication in macrophages in vitro. In intracranial tumors, RAMBO demonstrated significantly more viral gene expression than rHSVQ. Corroborating the study of Meisen et al., this group found a similar contribution of TNFα expression to OVT efficacy. Specifically in the presence of anti-TNFα antibody, rHSVQ1 replicated to levels similar to RAMBO in glioma cells co-cultured with macrophage, indicating that TNFα plays a large contribution in limiting the replication of rHSVQ1 in this system.

In a xenograft sarcoma model (A673) where the immunosuppressive TME is characterized by the infiltration and activation of M2 macrophage, Denton et al., [46] found that removing these cells via clodrosome or trabectedin resulted in greater efficacy of OVT (rRp450) with no accompanying increase in viral replication. M2 macrophages are major mediators of immunosuppression in this model [67]. While trabectedin did not reduce A673 growth as a monotherapy, in combination with OVT, trabectedin greatly reduced tumor growth and enhanced survival. Interestingly, the authors report that trabectedin reduced MDSCs and NK cells in addition to macrophages. These results suggest that OVT in combination with pharmaceuticals that modulate the cellular constituents of the tumor microenvironment, specifically macrophage polarization, may enhance the therapeutic potential of HSV-1 OVT.

Conclusions

Herpesviruses establish latent infection for the life of the host as part of their life cycle and therefore are expected to promote quantitatively and qualitatively different immune responses than acute pathogens such as adenoviruses or poxviruses. With their armament of immune modulatory gene products, herpesviruses orchestrate innate and adaptive immune responses to suit the establishment and maintenance of latent infection and subsequent reactivation to maintain the latent reservoir. Indeed, as lack of effective anti-viral T cell responses lead to unchecked virus replication, the development of anti-viral T cell responses can be seen as part of the life cycle of the herpes virus. In this sense, using herpesviruses as vectors for generating immune responses against infectious disease agents and tumors is a rational application of their unique biology. To take advantage of the biology of herpesvirus infection for such applications, a growing number of studies support the retention or even enhancement of immune evasion function to promote greater replication and spread in tumors.

It is a bit of a paradox that an immunosuppressed tumor microenvironment may be hostile to efficient viral replication.

However, suppression of anti-tumor immunity does not necessarily equal suppression of anti-viral immunity. A complex interplay of viral and host factors will need to be understood in order to generate more efficacious HSV-1 OVT. In the many cases of NK cell and macrophage depletion presented above, it is fair to ask—are we removing a population of cells that participates in mediating immunosuppression in the tumor microenvironment, or are we allowing the virus to better replicate and spread through the tumor? Likely both make contributions. Many of the depletion experiments described above reported an increase in viral replication in tumors and we can presume that this mediates, in part, the enhanced efficacy of treatment. However, Denton et al. did not report an increase in viral replication after depletion of macrophages or NK cells in their sarcoma model. In this case, perhaps reduction of immunosuppression in the tumor microenvironment in addition to enhanced immunogenicity is the more important factor contributing to the development of anti-tumor responses.

Interestingly, most of the studies of the kind reported here involve the subversion of innate immune responses to restore or enhance efficacy of HSV-1 OVT. In the cases of glioblastoma xenografts in athymic mice, the enhancement of efficacy can only be due to innate immunity rather than the development of an anti-tumor T cell response. However, the efficacy of most immunotherapies is CD8+ T cell mediated. Therefore, a focus on mechanisms that facilitate the development of cell-mediated anti-tumor immunity is expected to make an outsized contribution to the translational potential of this work. In this respect, it has been reported that NK cells can impair the development of anti-viral T cell responses including both effector and memory T cells [68]. Perhaps in some cancers, subversion of NK cell immunity in combination with OVT will improve the development of anti-tumor T cell responses.

We anticipate that next-generation HSV-1 oncolytic virotherapies will focus on maintaining much of the replicative potential of HSV-1 while limiting its ability to spread to neurons where they can establish latency, potentially recombine with wild-type virus strains, and subsequently reactivate to cause disease. As mentioned above, our lab has identified mutations that block entry of HSV-1 into neurons [29•]. This virus, VC2, has shown promise as an anti-HSV-1 and HSV-2 live-attenuated vaccine [30, 31, 69, 70]. VC2 maintains its replicative potential and in a mouse model of melanoma demonstrates significant efficacy as well as a potent ability to disrupt the tumor microenvironment (Rider et al. manuscript in preparation). Others have included mutations that disrupt transport in neurons [34•] in combination with miRNA target sites inserted into essential genes which precludes their expression in healthy tissues [71]. This virus has been reported to demonstrate potent anti-tumor activity in vivo [71].

It is becoming increasingly clear that understanding mechanisms of immunosuppression in the tumor microenvironment and the ways in which oncolytic herpesviruses affect and are affected by this environment will be important to informing the rational design of next-generation oncolytic viruses. Adding complexity, it is important to recognize that the diversity of cancers and the mechanisms of immunosuppression within those cancers makes generalization difficult. As such, there is likely not a "one size fits all" solution with oncolytic virotherapy. The support of studies detailing mechanisms of immunosuppression for individual malignancy types will be critical for aiding in the selection and design of HSV OVT vectors.

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Compliance with Ethical Standards

Conflict of Interest Dr. Kousoulas reports non-financial support from Ios Biomedical Group, Inc. (IBG), outside the submitted work; in addition, Dr. Kousoulas has a patent "vaccines against genital herpes infections" (Patent number: 10130703) licensed to Ios Biomedical Group, Inc., and a patent "synthetic herpes simplex viruses type-1 for treatment of cancers" (Patent number: 8586028) issued.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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